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2 **WHAT IS CLAIMED IS:**

1 1. An isolated polypeptide comprising a mutant peptide sequence,
2 wherein the mutant peptide sequence encodes an O-linked glycosylation site that does not
3 exist in a wild-type polypeptide corresponding to the isolated polypeptide.

1 2. The polypeptide of claim 1, wherein the polypeptide is a G-CSF
2 polypeptide.

1 3. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of M^1X_nTPLGP or $M^1B_oPZ_mX_nTPLGP$, and
3 wherein

4 the superscript denotes the position of the amino acid in the wild-type G-CSF
5 amino acid sequence (SEQ ID NO:3), the subscripts n and m are integers selected from 0 to
6 3, and

7 at least one of X and B is Thr or Ser, and

8 when more than one of X and B is Thr or Ser, the identity of these moieties is
9 independently selected, and

10 Z is selected from glutamate, or any uncharged amino acid.

1 4. The mutant G-CSF polypeptide of claim 3, wherein the mutant peptide
2 sequence is selected from the sequences consisting of MVTPPLGP, MQTPLGP,
3 MIATPLGP), MATPLGP, MPTQGAMPLGP , MVQTPPLGP, MQSTPLGP,
4 MGQTPPLGP, MAPTSSSPLGP, and MAPTPLGPA.

1 5. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of $M^1TPX_nB_oO_rP$
3 wherein

4 the superscript denotes the position of the amino acid in SEQ ID NO:3, and

5 the subscripts n, o, and r are integers selected from 0 to 3, and

6 at least one of X, B and O is Thr or Ser, and

7 when more than one of X, B and O is Thr or Ser, the identity of these moieties

8 is independently selected.

1 6. The polypeptide of claim 5, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: MTPTLGP, MTPTQLGP, MTPTSLGP,
3 MTPTQGP, MTPTSSP, M¹TPQTP, M¹TPTGP, M¹TPLTP, M¹TPNTGP, MTPLGP (G-
4 CSF mut #4), M¹TPVTP, M¹TPMVTP, and MT¹P²TQGL³G⁴P⁵A⁶S⁷.

1 7. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of LGX⁵³B_oLGI
3 wherein

4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO: 3), and
6 X is histidine, serine, arginine, glutamic acid or tyrosine, and
7 B is either threonine or serine, and
8 o is an integer from 0 to 3.

1 8. The polypeptide of claim 7, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: LGHTLGI, LGSSLGI, LGYSLGI, LGESLGI,
3 and LGSTLGI.

1 9. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of P¹²⁹Z_mJ_qO_rX_nPT
3 wherein

4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO. 3),
6 Z, J, O and X are independently selected from Thr or Ser, and
7 m, q, r, and n are integers independently selected from 0 to 3..

1 10. The polypeptide of claim 9, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: P¹²⁹ATQPT, P¹²⁹TLGPT, P¹²⁹TQGPT,
3 P¹²⁹TSSPT, P¹²⁹TQGAPT, P¹²⁹NTGPT, PALQPTQT, P¹²⁹ALTPT, P¹²⁹MVTPT,
4 P¹²⁹ASSTPT, P¹²⁹TTQP, P¹²⁹NTLP, P¹²⁹TLQP, MAP¹²⁹ATQPTQGAM, and
5 MP¹²⁹ATTQPTQGAM.

1 11. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of PZ_mU_sJ_qP⁶¹O_rX_nB_oC
3 wherein

4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO. 3),
6 at least one of Z, J, O, and U is selected from threonine or serine, and
7 when more than one of Z, J, O and U is threonine or serine, each is
8 independently selected, and
9 m, s, q, r, n, and o are integers independently selected from 0 to 3.

1 12. The polypeptide of claim 11, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: P⁶¹TSSC, P⁶¹TSSAC, LGIPTA P⁶¹LSSC,
3 LGIPTQ P⁶¹LSSC, LGIPTQG P⁶¹LSSC, LGIPQT P⁶¹LSSC, LGIPTS P⁶¹LSSC, LGIPTS
4 P⁶¹LSSC, LGIPTQP⁶¹LSSC, LGTPWAP⁶¹LSSC, LGTPFA P⁶¹LSSC, P⁶¹FTP, and
5 SLGAP⁵⁸TAP⁶¹LSS.

1 13. The polypeptide of claim 2, wherein the G-CSF polypeptide comprises
2 a mutant peptide sequence with the formula of $\emptyset_a G_p J_q O_r P^{175} X_n B_o Z_m U_s \Psi_t$
3 wherein

4 the superscript denotes the position of the amino acid in the wild type G-CSF
5 amino acid sequence (SEQ ID NO. 3),
6 at least one of Z, U, O, J, G, \emptyset , B and X is threonine or serine, and when more
7 than one of Z, U, O, J, G, \emptyset , B and X are threonine or serine, they are
8 independently selected; \emptyset is optionally R, and G is optionally H; the symbol Ψ
9 represents any uncharged amino acid residue or glutamate and
10 a, p, q, r, n, o, m, s, and t are integers independently selected from 0 to 3..

1 14. The polypeptide of claim 13, wherein the mutant peptide sequence is
2 selected from the sequences consisting of: RHLAQTP¹⁷⁵, RHLAGQTP¹⁷⁵,
3 QP¹⁷⁵TQGAMP, RHLAQTP¹⁷⁵AM, QP¹⁷⁵TSSAP, QP¹⁷⁵TSSAP, QP¹⁷⁵TQGAMP,
4 QP¹⁷⁵TQGAM, QP¹⁷⁵TQGA, QP¹⁷⁵TVM, QP¹⁷⁵NTGP, and QP¹⁷⁵QTLP.

1 15. The polypeptide of claim 2, comprises a mutant peptide sequence
2 selected from the sequences P¹³³TQTAMP¹³⁹, P¹³³TQGTMP, P¹³³TQGTNP,
3 P¹³³TQGTLP, and PALQP¹³³TQTAMPA.

1 16. The polypeptide of claim 1, wherein the polypeptide is an hGH
2 polypeptide.

1 17. The polypeptide of claim 16, wherein the mutant peptide sequence
2 comprises a sequence selected from: M¹APRSSPTIPL⁷SR⁹ and DGSP¹³³NTGQIFK¹⁴⁰

1 18. The polypeptide of claim 15, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of P¹³³JXBOZUK¹⁴⁰QTYS, and

3 wherein

4 the superscript denotes the position of the amino acid in the wild type hGH
5 amino acid sequence (SEQ ID NO: 20), and

6 J is selected from threonine and arginine;

7 X is selected from alanine, glutamine, isoleucine, and threonine;

8 B is selected from glycine, alanine, leucine, valine, asparagine, glutamine, and
9 threonine;

10 O is selected from tyrosine, serine, alanine, and threonine;

11 Z is selected from isoleucine and methionine; and

12 U is selected from phenylalanine and proline.

1 19. The polypeptide of claim 18, wherein the mutant peptide sequence is
2 selected from the group consisting of PTTGQIFK, PTTAQIFK, PTTLQIFK,
3 PTLYVFK, PTTVQIFK, PTTVSIFK, PTTNQIFK, PTTQQIFK, PTATQIFK,
4 PTQGQIFK, PTQGAIFK, PTQGAMFK, PTIGQIFK, PTINQIFK, PTINTIFK,
5 PTILQIFK, PTIVQIFK, PTIQQIFK, PTIAQIFK, P¹³³TTTQIFK¹⁴⁰QTYS, and
6 P¹³³TQGAMPK¹⁴⁰QTYS.

1 20. The polypeptide of claim 15, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of P¹³³RTGQIPTQBYS

3 wherein

4 the superscript denotes the position of the amino acid in the wild type hGH
5 amino acid sequence (SEQ ID NO:20), and

6 B is selected from alanine and threonine.

1 21. The polypeptide of claim 20, wherein the mutant peptide sequence is
2 selected from the group consisting of PRTGQIPTQTYS and PRTGQIPTQAYS.

1 22. The polypeptide of claim 16, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of L¹²⁸XTBOP¹³³UTG

3 wherein

4 superscripts denote the position of the amino acid in the wild-type hGH amino
5 acid sequence; and wherein
6 X is selected from glutamic acid, valine and alanine;
7 B is selected from glutamine, glutamic acid, and glycine;
8 O is selected from serine and threonine; and
9 U is selected from arginine, serine, alanine and leucine

1 23. The mutant hGH polypeptide of claim 22, wherein the mutant peptide
2 sequence is selected from the group consisting of: LETQSP¹³³RTG, LETQSP¹³³STG,
3 LETQSP¹³³ATG, LETQSP¹³³LTG, LETETP¹³³R, LETETP¹³³A, LVTQSP¹³³RTG,
4 LVTETP¹³³RTG, LVTETP¹³³ATG, and LATGSP¹³³RTG.

1 24. The polypeptide of claim 16, wherein the hGH polypeptide comprises
2 a mutant peptide sequence with a formula of M¹BPTX_nZ_mOPLSRL

3 wherein

4 wherein the superscript denotes the position of the amino acid in the wild type
5 hGH amino acid sequence (SEQ ID NO:19); and
6 B is selected from phenylalanine, valine and alanine or a combination thereof;
7 X is selected from glutamate, valine and proline
8 Z is threonine;
9 O is selected from leucine and isoleucine; and
10 when X is proline, Z is threonine; and

11 wherein

12 n and m are integers selected from 0 and 2.

1 25. The polypeptide of claim 24, wherein the mutant peptide sequence is
2 selected from the group consisting of M¹FPTE IPLSRL, M¹FPTV LPLSRL, and
3 M¹APPTIPLSRL.

1 26. The polypeptide of claim 24, wherein the mutant peptide sequence is
2 M¹VTPTIPLSRL, wherein the superscript 1, denotes the first position amino acid in the
3 wild type hGH amino acid sequence (SEQ ID NO:19)

1 27. The polypeptide of claim 15, wherein the mutant peptide sequence is
2 selected from the group consisting of: LEDGSPTTGQIFKQTYs,

3 LEDGSPTTAQIFKQTYS, LEDGSPTATQIFKQTYS, LEDGSPTQGAMFKQTYS,
4 LEDGSPTQGAIFKQTYS, LEDGSPTQGQIFKQTYS, LEDGSPTTLYVFKQTYS,
5 LEDGSPTINTIFKQTYS, LEDGSPTTVSIFKQTYS, LEDGSPRTGQIPTQTYS,
6 LEDGSPRTGQIPTQAYS, LEDGSPTTLQIFKQTYS, LETETPRTGQIFKQTYS,
7 LVTETPRTGQIFKQTYS, LETQSPRTGQIFKQTYS, LVTQSPRTGQIFKQTYS,
8 LVTETPATGQIFKQTYS, LEDGSPTQGAMPKQTYS, and LEDGSPTTQIFKQTYS.

1 28. The polypeptide of claim 1, wherein the polypeptide is an IFN alpha
2 polypeptide.

1 29. The polypeptide of claim 28, wherein wherein the INF alpha
2 polypeptide has a peptide sequence comprising a mutant amino acid sequence, and the
3 peptide sequence corresponds to a region of INF alpha 2 having a sequence as shown in
4 SEQ NO:22, and wherein the mutant amino acid sequence contains a mutation to a
5 threonine or serine amino acid at a position corresponding to T¹⁰⁶ of INF alpha 2.

1 30. The polypeptide of claim 29, wherein the IFN alpha polypeptide is
2 selected from the group consisting of IFN alpha, IFN alpha 4, IFN alpha 5, IFN alpha 6,
3 IFN alpha 7, IFN alpha 8, IFN alpha 10, IFN alpha 14, IFN alpha 16, IFN alpha 17, and
4 IFN alpha 21.

1 31. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha polypeptide comprising a mutant amino acid sequence selected from the group
3 consisting of:

4 ⁹⁹CVMQEERVVTETPLMNADSI¹¹⁸, ⁹⁹CVMQEEGVVTETPLMNADSI¹¹⁸,
5 and ⁹⁹CVMQGVGVVTETPLMNADSI¹¹⁸.

1 32. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 4 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGVVTETPLMNVDI¹¹⁸, and ⁹⁹CVIQGVGVVTETPLMKEDSI¹¹⁸.

1 33. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 5 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CMMQEVGVTDTPLMVDSIL}^{118}$, $^{99}\text{CMMQEVGVTETPLMVDSIL}^{118}$
 5 and $^{99}\text{CMMQGVGVTDPLMVDSIL}^{118}$.

1 34. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 6 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CVMQE}\text{VWVTGTP}\text{LMNEDSIL}^{118}$, $^{99}\text{CVMQE}\text{VGVTGTP}\text{LMNEDSIL}^{118}$,
 5 and $^{99}\text{CVMQGVGV}\text{TETPLMNEDSIL}^{118}$.

1 35. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 7 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CVIQEVGVETPLMNEDFIL}^{118}$, and $^{99}\text{CVIQGVGVETPLMNEDFIL}^{118}$.

1 36. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 8 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CVMQEVGVTESPLMYEDSIL}^{118}$, and $^{99}\text{CVMQGVGVTEESPLMYEDSIL}^{118}$.

1 37. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 10 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CVIQEVGVETPLMNEDSIL}^{118}$, and $^{99}\text{CVIQGVGVETPLMNEDSIL}^{118}$.

1 38. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 14 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 $^{99}\text{CVIQEVGVETPLMNEDSIL}^{118}$, and $^{99}\text{CVIQGVGVETPLMNEDSIL}^{118}$.

1 39. The polypeptide of claim 30, wherein the IFN alpha polypeptide is an
2 IFN alpha 16 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

⁹⁹CVTQEVGVTEIPLMNEDSIL¹¹⁸, ⁹⁹CVTQEVGVETPLMNEDSIL¹¹⁸, and ⁹⁹CVTQGVGVETPLMNEDSIL¹¹⁸.

1 40. The polypeptide of claim **30**, wherein the IFN alpha polypeptide is an
2 IFN alpha 17 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGMTETPLMNEDSIL¹¹⁸, ⁹⁹CVIQEVGVETPLMNEDSIL¹¹⁸, and
5 ⁹⁹CVIQGVGMTETPLMNEDSIL¹¹⁸.

1 41. The polypeptide of claim **30**, wherein the IFN alpha polypeptide is an
2 IFN alpha 21 polypeptide comprising a mutant amino acid sequence selected from the
3 group consisting of:

4 ⁹⁹CVIQEVGVETPLMNVD SIL¹¹⁸, and ⁹⁹CVIQGVGVETPLMNVD SIL¹¹⁸.

1 42. An isolated nucleic acid encoding the polypeptide of claim 1.

1 43. An expression cassette comprising the nucleic acid of claim **42**.

1 44. A cell comprising the nucleic acid of claim **42**.

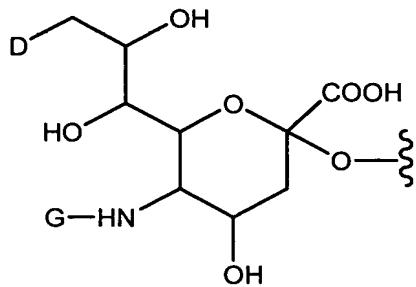
1 45. The polypeptide of claim 1, having a formula selected from:



3 wherein AA is an amino acid a side chain that comprises a hydroxyl moiety
4 that is within the mutant peptide sequence; and X a modifying group or a saccharyl moiety.

1 46. The polypeptide according to claim **45**, wherein X comprises a group
2 selected from sialyl, galactosyl and Gal-Sia moieties, wherein at least one of said sialyl,
3 galactosyl and Gal-Sia comprises a modifying group.

1 47. The polypeptide according to claim **45**, wherein X comprises the
2 moiety:



wherein

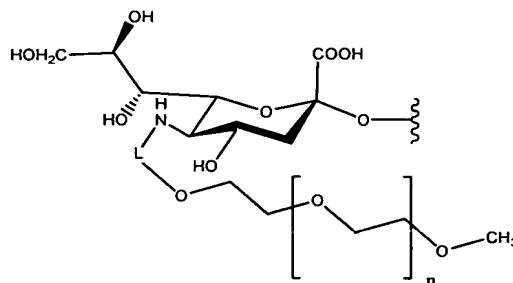
5 D is a member selected from -OH and R¹-L-HN-;

6 G is a member selected from R¹-L- and -C(O)(C₁-C₆)alkyl;

7 R¹ is a moiety comprising a member selected a moiety comprising a straight-
8 chain or branched poly(ethylene glycol) residue; and

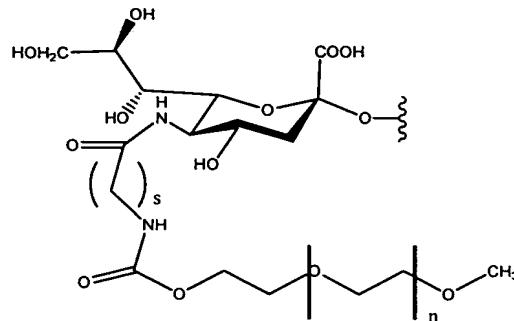
9 L is a linker which is a member selected from a bond, substituted or
10 unsubstituted alkyl and substituted or unsubstituted heteroalkyl,
11 such that when D is OH, G is R¹-L-, and when G is -C(O)(C₁-C₆)alkyl, D is
12 R¹-L-NH-.

1 48. The polypeptide according to claim 45, wherein X comprises the
2 structure:



4 in which L is a substituted or unsubstituted alkyl or substituted or unsubstituted
5 heteroalkyl group; and n is selected from the integers from 0 to about 500.

1 49. The polypeptide according to claim 45, wherein X comprises the
2 structure:



wherein s is selected from the integers from 0 to 20.

50. A method for making a glycoconjugate of the polypeptide of claim 1, comprising the steps of:

- (a) recombinantly producing the polypeptide, and
- (b) enzymatically glycosylating the polypeptide with a modified sugar at glycosylation site.

51. A pharmaceutical composition of a granulocyte colony stimulating factor (G-CSF) comprising: an effective amount of the polypeptide of claim 2, wherein said polypeptide is glycoconjugated with a modified sugar.

52. The pharmaceutical composition according to claim 51, wherein said modified sugar is modified with a member selected from poly(ethylene glycol) and methoxy-poly(ethylene glycol) (m-PEG).

53. A pharmaceutical composition of human Growth Hormone (hGH) comprising an effective amount of the polypeptide of claim 16, wherein said polypeptide is glycoconjugated with a modified sugar.

54. The pharmaceutical composition according to claim 53, wherein said modified sugar is modified with a member selected from poly(ethylene glycol) and methoxy-poly(ethylene glycol) (m-PEG).

55. A pharmaceutical composition of a granulocyte macrophage colony stimulating factor (GM-CSF) comprising an effective amount of GM-CSF polypeptide comprising a mutant peptide sequence, wherein the mutant sequence comprises an O-linked glycosylation site that does not exist in a wild-type GM-CSF polypeptide, and wherein said polypeptide is glycoconjugated with a modified sugar.

1 56. The pharmaceutical composition according to claim 55, wherein said
2 modified sugar is modified with a member selected from poly(ethylene glycol) and
3 methoxy-poly(ethylene glycol) (m-PEG).

1 57. A pharmaceutical composition of an interferon alpha-2b comprising an
2 effective amount of the polypeptide of claim 28, wherein said polypeptide is
3 glycoconjugated with a modified sugar.

1 58. The pharmaceutical composition according to claim 57, wherein said
2 modified sugar is modified with a member selected from poly(ethylene glycol) and
3 methoxy-poly(ethylene glycol) (m-PEG).

1 59. A method of providing G-CSF therapy to a subject in need of said
2 therapy, said method comprising, administering to said subject an effective amount the
3 pharmaceutical composition of claim 51.

1 60. A method of providing granulocyte macrophage colony stimulating
2 factor therapy to a subject in need of said therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 55.

1 61. A method of providing interferon therapy to a subject in need of said
2 therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 57.

1 62. A method of providing Growth Hormone therapy to a subject in need
2 of said therapy, said method comprising:
3 administering to said subject an effective amount the pharmaceutical
4 composition of claim 53.